09734039 21535418 PMID: 11680719

Optimized T7 amplification system for microarray analysis.

Pabon C; Modrusan Z; Ruvolo M V; Coleman I M; Daniel S; Yue H; Arnold L J
Incyte Genomics, Palo Alto, CA 94304, USA.

BioTechniques (United States) Oct 2001, 31 (4) p874-9, ISSN 0736-6205 Journal Code: 8306785
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Glass cDNA microarray technologies offer a highly parallel approach for profiling expressed gene sequences in disease-relevant tissues. However, standard hybridization and detection protocols are insufficient for milligram quantities of tissue, such as those derived from needle biopsies. Amplification systems utilizing T7 RNA polymerase can provide multiple **cRNA** copies from mRNA transcripts, permitting microarray studies with reduced sample inputs. Here, we describe an optimized T7-based amplification system for microarray analysis that yields between 200- and 700-fold amplification. This system was evaluated with both **mRNA** and **total** RNA samples and provided microarray sensitivity and precision that are comparable to our standard production process without amplification. The size distributions of amplified **cRNA** ranged from 200 bp to 4 kb and were similar to original mRNA profiles. These amplified **cRNA** samples were fluorescently labeled by reverse transcription and hybridized to microarrays comprising approximately 10,000 cDNA targets using a dual-channel format. Replicate hybridization experiments were conducted with the same and different tissues in each channel to assess the sensitivity and precision of differential expression ratios. Statistical analysis of differential expression ratios showed the lower limit of detection to be about 2-fold within and between amplified data sets, and about 3-fold when comparing amplified data to unamplified data (99.5% confidence).

```
? ds

Set     Items   Description
S1       5626   CRNA
S2    1250300   REPRESENT?
S3        360   S1 AND S2
S4     457890   MRNA
S5        175   S3 AND S4
S6         89   RD (unique items)
S7       3636   TOTAL(2N)MRNA
S8          9   S3 AND S7
S9          4   RD (unique items)
? review
>>>none
? s review
     S10  682927  REVIEW
? s s1 and s10
           5626  S1
         682927  S10
     S11      24  S1 AND S10
? rd
>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
     S12      16  RD (unique items)
? t s12/3,k,ab/1-16
```

Application of genome-wide gene expression profiling by high-density DNA arrays to the treatment and study of inflammatory bowel disease.

Warner Elaine E; Dieckgraefe Brian K

Division of Gastroenterology, Washington University School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110, U.S.A.

Inflammatory bowel diseases (United States) Mar 2002, 8 (2) p140-57, ISSN 1078-0998 Journal Code: 9508162

Contract/Grant No.: AI 48137; AI; NIAID; P30 DK 52574; DK; NIDDK

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Identification of factors involved in the initiation, amplification, and perpetuation of the chronic immune response and the identification of markers for the characterization of patient subgroups remain critical objectives for ongoing research in inflammatory bowel disease (IBD). The Human Genome Project and the development of the expressed sequence tag (EST) clone collection and database have made possible a new revolution in gene expression analysis. Instead of measuring one or a few genes, parallel DNA microarrays are capable of simultaneously measuring expression of thousands of genes, providing a glimpse into the logic and functional grouping of gene programs encoded by our genome. Applied to clinical specimens from affected and normal individuals, this methodology has the potential to provide a new level of information about disease pathogenesis not previously possible. Two dominant platforms for the construction of high-density microarrays have emerged: cDNA arrays and GeneChips. The first involves robotic spotting of DNA molecules, often derived from EST clone collections, onto a suitable solid phase matrix such as a glass slide. The second involves direct in situ synthesis of sets of gene-specific oligonucleotides on a silicon wafer by an eloquent derivative of the photolithography process. Both cDNA and oligonucleotide arrays are interrogated by hybridization with a fluorescent-labeled cDNA or **cRNA** representation of the original tissue mRNA. This enables measurement of the expression levels for thousands of mucosal genes in a single experiment. These technologies have recently become less expensive and more widely accessible to all researchers. This **review** details the principles and methods behind DNA array technology, data analysis and mining, and

9/3,K,AB/4 (Item 1 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US Patent

Dialog Acc No: 3554024 IFI Acc No: 0129683
Document Type: C
RNA AMPLIFICATION METHOD; A POLYMERASE CHAIN REACTION KIT FOR INCORPORATING AN RNA POLYMERASE PROMOTER INTO DOUBLE STRANDED CDNA BY AMPLIFICATION WITH AN RNA PRIMER FOR LESS THAN 20 CYCLES; THEN THE RNA POLYMERASE CONTAINING CDNA IS THEN TRANSCRIBED INTO RNA
Inventors: Linsley Peter S (US); Schelter Janell M (US)
Assignee: Rosetta Inpharmatics Inc
Assignee Code: 51113
Publication (No,Date), Applic (No,Date):
US 6271002 20010807 US 99411074 19991004
Publication Kind: B
Calculated Expiration: 20191004
Priority Applic(No,Date): US 99411074 19991004

Abstract: The present invention relates to methods and kits for amplification of mRNA using a primer in PCR that contains an RNA polymerase promoter. The invention provides methods for amplification and detection of RNA derived from a population of cells, preferably eukaryotic cells and most preferably mammalian cells, which methods preserve fidelity with respect to sequence and transcript **representation**, and additionally enable amplification of extremely small amounts of mRNA, such as might be obtained from 106 mammalian cells. In typical embodiments of the invention, an RNA polymerase promoter (RNAP) is incorporated into ds cDNA by priming cDNA amplification by polymerase chain reaction (PCR) with an RNAP-containing primer. Following less than 20 cycles of PCR, the resultant RNAP-containing ds cDNA is transcribed into RNA using an RNA polymerase capable of binding to the RNAP introduced during cDNA synthesis. This combination of PCR and in vitro transcription (IVT) enables the generation of a relatively large amount of RNA from a small starting number of cells without loss of fidelity. RNAs generated using this method may be labeled and employed to profile gene expression in different populations of cells, e.g., by use of a polynucleotide microarray.

Abstract: ...and most preferably mammalian cells, which methods preserve fidelity with respect to sequence and transcript **representation**, and additionally enable amplification of extremely small amounts of mRNA, such as might be obtained...

Exemplary Claim: ...polymerase promoter sequence in sense or antisense orientation; and (d) transcribing resultant amplified DNA into **cRNA** by contacting the amplified DNA with an RNA polymerase specific for said RNA polymerase promoter sequence introduced in step (c) under conditions conducive to RNA polymerase activity, such that **cRNA** is produced.
Non-exemplary Claims: ...19. The method of claim 1 wherein said sample contains total RNA or **total mRNA** from cells...

...method of claim 2, 3, 15 or 16 wherein said sample contains total RNA or **total mRNA** from cells...30. The method of claim 1, which further comprises labeling the transcribed **cRNA** with a label...

...34. The method of claim 32, wherein a first aliquot of the **cRNA** is labeled with a first fluorophore having a first emission spectrum, and a second aliquot of the **cRNA** is labeled with a second fluorophore with a second emission spectrum differing from that of...mRNA is extracted from at least one cell of interest, and further comprising contacting the **cRNA** produced in step (d) with an array containing

one or more species of polynucleotide positioned...
...detecting any hybridization that occurs between said one or more species of polynucleotide and said **cRNA**.
...

...mRNA is extracted from at least one cell of interest, and further comprising contacting the **cRNA** produced in step (d) with an array containing one or more species of polynucleotide positioned...

...detecting any hybridization that occurs between said one or more species of polynucleotide and said **cRNA**.
...

...mRNA is extracted from at least one cell of interest, and further comprising contacting the **cRNA** produced in step (d) with an array containing one or more species of polynucleotide positioned...

...detecting any hybridization that occurs between said one or more species of polynucleotide and said **cRNA**.
...

...RNA polymerase promoter sequence in sense or antisense orientation; (d) transcribing resultant amplified DNA into **cRNA** by contacting the amplified DNA with an RNA polymerase specific for said RNA polymerase promoter sequence introduced in step (c) under conditions conducive to RNA polymerase activity, such that **cRNA** is produced; (e) labeling the **cRNA** produced in step (d) with a first label; (f) repeating steps (a)-(d) with said second sample; (g) labeling the **cRNA** produced in step (f) with a second label distinguishable from said first label; (h) detecting or measuring the mRNA of interest in the first sample by contacting the **cRNA** labeled with said first label with a polynucleotide probe capable of hybridizing to said **cRNA** of the mRNA of interest ...conditions conducive to hybridization; and detecting any hybridization that occurs between said probe and said **cRNA**; (i) detecting or measuring the mRNA of interest in the second sample by contacting the **cRNA** labeled with said second label with said polynucleotide probe capable of hybridizing to said **cRNA** of the mRNA of interest under conditions conducive to hybridization; and detecting any hybridization that occurs between said probe and said **cRNA**; and (j) comparing the mRNA of interest detected or measured in said first sample with...63. The method of claim 45 wherein said sample contains total RNA or **total mRNA** from cells...

...64. The method of claim 46 wherein said sample contains total RNA or **total mRNA** from cells...

...65. The method of claim 59 or 60 wherein said sample contains total RNA or **total mRNA** from cells...or measuring steps (h) and (i) are carried out by a method comprising contacting said **cRNA** with an array containing one or more species of polynucleotide probe positioned at preselected sites...

...conducive to hybridization; and detecting any hybridization that occurs between said polynucleotide probes and said **cRNA**.
...

...or measuring steps (h) and (i) are carried out by a method comprising contacting said **cRNA** with an array containing one or more species of polynucleotide probe positioned at preselected sites...

...conducive to hybridization; and detecting any hybridization that occurs between said polynucleotide probes and said **cRNA**.
...

...or measuring steps (h) and (i) are carried out by a method comprising contacting said **cRNA** with an array containing one or more species of polynucleotide probe positioned at preselected sites...

...conducive to hybridization; and detecting any hybridization that occurs between said polynucleotide probes and said **cRNA**.

...claim 45 or 46 wherein in steps (h) and (i), the steps of contacting the **cRNA** labeled with said first label with said polynucleotide probe, and contacting the **cRNA** labeled with said second label with said polynucleotide probe, are carried out concurrently...

...claim 59 or 60 wherein in steps (h) and (i), the steps of contacting the **cRNA** labeled with said first label with said polynucleotide probe, and contacting the **cRNA** labeled with said second label with said polynucleotide probe, are carried out concurrently...

```
? ds

Set     Items   Description
S1       5626   CRNA
S2    1250300   REPRESENT?
S3        360   S1 AND S2
S4     457890   MRNA
S5        175   S3 AND S4
S6         89   RD (unique items)
S7       3636   TOTAL(2N)MRNA
S8          9   S3 AND S7
S9          4   RD (unique items)
S10    682927   REVIEW
S11        24   S1 AND S10
S12        16   RD (unique items)
? s array??
     S13  292917  ARRAY??
? s s1 and s13
           5626  S1
         292917  S13
     S14     120  S1 AND S13
? s s14 and s7
            120  S14
           3636  S7
     S15       3  S14 AND S7
? rd
>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
     S16       2  RD (unique items)
? t s16/3,k,ab/1-2
```

```
? ds

Set     Items   Description
S1       9664   IN(W)VITRO(W)TRANSCRIPTION
S2    1284900   LIMITATION? OR DISADVANTAGE? OR PROBLEM??
S3         91   S1 AND S2
S4         58   RD (unique items)
? s crna
      S5    5626  CRNA
? s s4 and s5
              58  S4
            5626  S5
      S6       0  S4 AND S5
? s limitation?? or disadvantage?
          184746  LIMITATION??
           49825  DISADVANTAGE?
      S7  232935  LIMITATION?? OR DISADVANTAGE?
? s s1 and s5
            9664  S1
            5626  S5
      S8     118  S1 AND S5
? s s8 and s7
             118  S8
          232935  S7
      S9       0  S8 AND S7
? s problem??
     S10 1076340  PROBLEM??
? s s8 and s10
             118  S8
         1076340  S10
     S11       0  S8 AND S10
? s in(w)vitro(w)transcription(5n)(problem?? or disadvantage? or limitation??)
Processing
Processing
Processing
Processing
Processing
Processing
        29015143  IN
         1372759  VITRO
          575460  TRANSCRIPTION
         1076340  PROBLEM??
           49825  DISADVANTAGE?
          184746  LIMITATION??
     S12       4  IN(W)VITRO(W)TRANSCRIPTION(5N)(PROBLEM?? OR DISADVANTAGE?
                  OR LIMITATION??)
? rd
>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
     S13       2  RD (unique items)
? t s13/3,k,ab/1-2

 13/3,K,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

08555841   95244141   PMID: 7727125
  Rapid  screening  of  open reading frames by protein synthesis with an in
vitro transcription and translation assay.
  Switzer W M; Heneine W
  Retrovirus  Diseases  Branch, Centers for Disease Control and Prevention,
Atlanta, GA 30333, USA.
```

BioTechniques (UNITED STATES) Feb 1995, 18 (2) p244-8, ISSN 0736-6205 Journal Code: 8306785
Document type: Technical Report
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The analysis of open reading frames (ORFs) to predict full-length or truncated proteins in genes is conventionally achieved by DNA sequencing. This method becomes labor-intensive when a large number of specimens or when large genes are to be examined. To circumvent this **problem**, we used an **in vitro transcription** and translation (TT) assay to identify full-length or truncated proteins in PCR-amplified genes. A total of 47 nef genes from the simian immunodeficiency virus (SIV) were cloned from 13 SIV-infected monkeys and were screened for ORFs by using the TT assay. Of these 47 genes, 20 had an intact ORF and 27 had premature stop codons at variable positions in the nef gene. All 20 nef genes with intact ORFs produced full-length proteins, while truncated proteins of different sizes were synthesized from all 27 nef genes with premature stop codons. In addition, we validated a simplified TT protocol that allows the direct screening of ORFs from transformed bacterial colonies, thus eliminating the need for plasmid preparations. By being rapid, simple and cost-effective, this technique should be widely applicable to examine the integrity of the ORF of any gene.

... large number of specimens or when large genes are to be examined. To circumvent this **problem**, we used an **in vitro transcription** and translation (TT) assay to identify full-length or truncated proteins in PCR-amplified genes...

```
? ds

Set     Items   Description
S1      14244   IN(W)VITRO(2N)TRANSCRIPTION
S2     429380   EFFICIENCY
S3        733   S1 AND S2
? s transcription(2n)efficiency
          574669  TRANSCRIPTION
          429380  EFFICIENCY
      S4    1046  TRANSCRIPTION(2N)EFFICIENCY
? s s1 and s4
           14244  S1
            1046  S4
      S5     182  S1 AND S4
? rd
>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...examined 50 records  (50)
...examined 50 records  (100)
...examined 50 records  (150)
...completed examining records
      S6     106  RD (unique items)
? t s6/3,k,ab/1-10

 6/3,K,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

14236766   22313517   PMID: 12213809
  Ethanol induction of class I alcohol dehydrogenase expression in the rat
occurs through alterations in CCAAT/enhancer binding proteins beta and
gamma.
  He Ling; Ronis Martin J J; Badger Thomas M; et al
  Arkansas Children's Nutrition Center, Little Rock 72202, USA.
  Journal of biological chemistry (United States)  09 03 2002,  277  (46)
 p43572-7,  ISSN 0021-9258  Journal Code: 2985121R
  Contract/Grant No.: AA0845; AA; NIAAA; +
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  Alcohol dehydrogenase (ADH) is the principal ethanol-metabolizing enzyme.
Ethanol induces rat Class I ADH mRNA and activity by an as yet unknown
mechanism. In the current study, adult male rats were fed an
ethanol-containing diet by continuous intragastric infusion for 42 days.
Hepatic Class I ADH mRNA, protein, and activity levels in the
ethanol-infused rats increased 3.9-, 3.3-, and 1.7-fold, respectively (p
<0.05). Cis-acting elements within the proximal promoter region of the ADH
gene were studied by electrophoretic mobility shift assay (EMSA). Hepatic
nuclear extract (HNE) binding to either the consensus or ADH-specific
CCAAT/enhancer binding protein (C/EBP) sites was >2.4-fold greater in
ethanol-fed rats (p <0.05) than controls. Antibody-specific EMSA assays
demonstrated binding of the transcription factor C/EBPbeta to the C/EBP
site. Western blot immunoblot analysis of HNEs demonstrated 3.5- and
2.3-fold increases in C/EBPbeta (LAP) and C/EBPdelta (p <0.05),
respectively, in ethanol-fed rats compared with controls, whereas levels of
the truncated C/EBPbeta (LIP) and C/EBPgamma were lower in ethanol-fed rats
(p <0.05). HNE from ethanol-fed rats increased (3-fold) the in
vitro transcription of rat Class I ADH (p <0.05), and mutation
of the C/EBP element in the proximal promoter region blocked this effect.
Antisera against LIP or C/EBPgamma enhanced transcription
efficiency (p <0.05). These data provide the first evidence for the
mechanism by which ethanol regulates rat hepatic Class I ADH gene
```

expression in vivo. This mechanism involves the C/EBP site and the enhancer binding proteins beta and gamma.

... ethanol-fed rats (p <0.05). HNE from ethanol-fed rats increased (3-fold) the **in vitro transcription** of rat Class I ADH (p <0.05), and mutation of the C/EBP element in the proximal promoter region blocked this effect. Antisera against LIP or C/EBPgamma enhanced **transcription efficiency** (p <0.05). These data provide the first evidence for the mechanism by which ethanol...

6/3,K,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11933385 99377166 PMID: 10446226
Bisanthracycline WP631 inhibits basal and Sp1-activated **transcription** initiation **in vitro**.
Martin B; Vaquero A; Priebe W; Portugal J
Departamento de Biologia Molecular y Celular, Instituto de Biologia Molecular de Barcelona, CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain.
Nucleic acids research (ENGLAND) Sep 1 1999, 27 (17) p3402-9, ISSN 1362-4962 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
An **in vitro transcription** assay was used to compare the capacity of the bisintercalating anthracycline WP631 (which displays a remarkably high DNA-binding affinity) and the monointercalating anthracycline daunomycin to inhibit transcription initiation of the adenovirus major late promoter linked to a G-less transcribed DNA template. Both drugs inhibit basal RNA synthesis in a concentration-dependent way, and the drug concentrations required to inhibit transcription initiation are similar. However, in this study WP631 was around 15 times more efficient at inhibiting transcription initiation when used with an adenovirus promoter containing an upstream Sp1-protein binding site under experimental conditions in which the Sp1 protein acted as a transactivator in vitro. The differences in the ability of each drug to inhibit transcription initiation were related to the competition between Sp1 and the drugs for the same binding site. Concentrations of WP631 as low as 60 nM could inhibit the Sp1-activated **transcription** initiation **in vitro** . In contrast, the concentration of daunomycin required to inhibit Sp1-activated transcription by 50% was almost the same as the concentration required to inhibit basal **transcription**. The **efficiency** of WP631 at displacing Sp1 from its putative binding site was confirmed using gel retardation and footprinting assays. These results are the first unequivocal example of a direct effect of an intercalator on activated transcription initiation.

Bisanthracycline WP631 inhibits basal and Sp1-activated **transcription** initiation **in vitro**.
An **in vitro transcription** assay was used to compare the capacity of the bisintercalating anthracycline WP631 (which displays a...

... binding site. Concentrations of WP631 as low as 60 nM could inhibit the Sp1-activated **transcription** initiation **in vitro** . In contrast, the concentration of daunomycin required to inhibit Sp1-activated transcription by 50% was almost the same as the concentration required to inhibit basal **transcription**. The **efficiency** of WP631 at displacing Sp1 from its putative binding site was confirmed using gel retardation...

6/3,K,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11921629 99365158 PMID: 10436020

The Werner syndrome protein is involved in RNA polymerase II transcription.

Balajee A S; Machwe A; May A; Gray M D; Oshima J; Martin G M; Nehlin J O; Brosh R; Orren D K; Bohr V A

Laboratory of Molecular Genetics, National Institute on Aging, National Institutes of Health, Baltimore, Maryland 21224, USA.

Molecular biology of the cell (UNITED STATES) Aug 1999, 10 (8) p2655-68, ISSN 1059-1524 Journal Code: 9201390

Contract/Grant No.: AG-01751; AG; NIA; AG-14446; AG; NIA; R24-CA-78088; CA; NCI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Werner syndrome (WS) is a human progeroid syndrome characterized by the early onset of a large number of clinical features associated with the normal aging process. The complex molecular and cellular phenotypes of WS involve characteristic features of genomic instability and accelerated replicative senescence. The gene involved (WRN) was recently cloned, and its gene product (WRNp) was biochemically characterized as a helicase. Helicases play important roles in a variety of DNA transactions, including DNA replication, transcription, repair, and recombination. We have assessed the role of the WRN gene in transcription by analyzing the **efficiency** of basal **transcription** in WS lymphoblastoid cell lines that carry homozygous WRN mutations. Transcription was measured in permeabilized cells by [3H]UTP incorporation and in vitro by using a plasmid template containing the RNA polymerase II (RNA pol II)-dependent adenovirus major late promoter. With both of these approaches, we find that the **transcription efficiency** in different WS cell lines is reduced to 40-60% of the transcription in cells from normal individuals. This defect can be complemented by the addition of normal cell extracts to the chromatin of WS cells. Addition of purified wild-type WRNp but not mutated WRNp to the **in vitro transcription** assay markedly stimulates RNA pol II-dependent transcription carried out by nuclear extracts. A nonhelicase domain (a direct repeat of 27 amino acids) also appears to have a role in transcription enhancement, as revealed by a yeast hybrid-protein reporter assay. This is further supported by the lack of stimulation of transcription when mutant WRNp lacking this domain was added to the in vitro assay. We have thus used several approaches to show a role for WRNp in RNA pol II transcription, possibly as a transcriptional activator. A deficit in either global or regional transcription in WS cells may be a primary molecular defect responsible for the WS clinical phenotype.

... recombination. We have assessed the role of the WRN gene in transcription by analyzing the **efficiency** of basal **transcription** in WS lymphoblastoid cell lines that carry homozygous WRN mutations. Transcription was measured in permeabilized...

... II)-dependent adenovirus major late promoter. With both of these approaches, we find that the **transcription efficiency** in different WS cell lines is reduced to 40-60% of the transcription in cells ...

...of WS cells. Addition of purified wild-type WRNp but not mutated WRNp to the **in vitro transcription** assay markedly stimulates RNA pol II-dependent transcription carried out by nuclear extracts. A nonhelicase...

6/3,K,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11897412 99340245 PMID: 10409736
In vitro analysis of alpha-amanitin-resistant transcription from the rRNA, procyclic acidic repetitive protein, and variant surface glycoprotein gene promoters in Trypanosoma brucei.
Laufer G; Schaaf G; Bollgonn S; Gunzl A
Abteilung Zellbiologie, Zoologisches Institut der Universitat Tubingen, D-72076 Tubingen, Germany.
Molecular and cellular biology (UNITED STATES) Aug 1999, 19 (8) p5466-73, ISSN 0270-7306 Journal Code: 8109087
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
In Trypanosoma brucei, transcription resistant to the mushroom toxin alpha-amanitin is not restricted to the rRNA genes (rDNA), as in higher eukaryotes, but extends to genes encoding the major cell surface proteins variant surface glycoprotein (VSG) and procyclin or procyclic acidic repetitive protein (PARP). Here, we report the development of a homologous cell extract from procyclic T. brucei cells in which rDNA and PARP A and VSG gene promoters drive efficient, accurate, and alpha-amanitin-resistant transcription. A comparative analysis revealed that transcription from the three promoters generally required identical reaction conditions for maximal efficiency. Nevertheless, PARP promoter transcription proved to be exceptional by its high efficiency, its lag phase, a high template DNA concentration optimum, and its tolerance to increasing concentrations of Mn(2+). Mutational analysis for both the PARP and rDNA promoters showed that the proximal and distal core elements were essential for efficient **transcription in vitro**. Deletion of the upstream control regions (UCRs), however, had a different effect. Whereas PARP UCR deletion reduced **transcription efficiency** almost 10-fold, deletion of the rDNA UCR had only a minor effect on **transcription efficiency**.

... and rDNA promoters showed that the proximal and distal core elements were essential for efficient **transcription in vitro**. Deletion of the upstream control regions (UCRs), however, had a different effect. Whereas PARP UCR deletion reduced **transcription efficiency** almost 10-fold, deletion of the rDNA UCR had only a minor effect on **transcription efficiency**.

6/3,K,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11527724 98418772 PMID: 9747976
Single nucleotide resolution of promoter activity and protein binding for the Leishmania tarentolae spliced leader RNA gene.
Yu M C; Sturm N R; Saito R M; Roberts T G; Campbell D A
Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90095-1747, USA.
Molecular and biochemical parasitology (NETHERLANDS) Aug 1 1998, 94 (2) p265-81, ISSN 0166-6851 Journal Code: 8006324
Contract/Grant No.: 2-T32-AI-07323; AI; NIAID; AI34536; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
In Kinetoplastid protozoa, trans-splicing is a central step in the

maturation of nuclear mRNAs. In Leishmania, a common 39 nt spliced-leader (SL) is transferred via trans-splicing from the precursor 96 nt SL RNA to the 5' terminus of all known protein-encoding RNAs. In this study, promoter elements of the L. tarentolae SL RNA gene have been identified with respect to transcriptional activity and putative transcription factor binding. We have mapped the essential regions in the SL RNA gene promoter at single nucleotide resolution using both in vivo **transcription** and **in vitro** protein/DNA binding approaches. Two regions located upstream of the SL RNA gene were identified: a GN3CCC element at -39 to -33 and a GACN5G element at -66 to -58 were essential for SL RNA gene transcription in stably transfected cells. Consistent with other known bipartite promoter elements, the spacing between the GN3CCC and GACN5G elements was found to be critical for proper promoter function and correct transcription start point selection, as was the distance between the two elements and the wild-type transcription start point. The GACN5G element interacts specifically and in a double-stranded form with a protein(s) in Leishmania nuclear extracts. The degree of this protein DNA interaction in vitro correlated with SL RNA gene **transcription efficiency** in vivo, consistent with a role of the protein as a transcription factor. The core nucleotides GACN5G fit the consensus PSE promoter structure of pol II-transcribed snRNA genes in metazoa.

... regions in the SL RNA gene promoter at single nucleotide resolution using both in vivo **transcription** and **in vitro** protein/DNA binding approaches. Two regions located upstream of the SL RNA gene were identified...

...extracts. The degree of this protein DNA interaction in vitro correlated with SL RNA gene **transcription efficiency** in vivo, consistent with a role of the protein as a transcription factor. The core...

6/3,K,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11198002 98074612 PMID: 9413132
Application of solution equilibrium analysis to **in vitro** RNA **transcription**.
Kern J A; Davis R H
Department of Chemical Engineering, University of Colorado, Boulder 80309-0424, USA.
Biotechnology progress (UNITED STATES) Nov-Dec 1997, 13 (6) p747-56, ISSN 8756-7938 Journal Code: 8506292
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Solution equilibrium analysis of **in vitro** RNA **transcription** has been applied to examine changes in pH, free magnesium concentration, and concentrations of all chemical ionization species as a transcription reaction proceeds. With this method, the progress of a transcription reaction can be accurately determined as a function of measured pH. In addition, it is demonstrated that this method has significant value as a tool for achieving improved understanding of the effects of varying solution conditions on the dynamics of RNA transcription. Magnesium concentration was found to be a critical factor for efficient transcription. Below 5 mM free Mg2+ concentration, the transcription rate and the efficiency at which nucleoside triphosphates (NTPs) are incorporated are greatly reduced. While inorganic pyrophosphate (PPi), a byproduct of the reaction, was found to directly inhibit the rate of transcription, its detrimental effects on transcription were determined to be primarily due to sequestering of magnesium. The PPi forms a precipitate with magnesium which was determined to have a molar composition

of 2:1 of Mg:PPi. **Transcription** rate and **efficiency** of NTP incorporation are also reduced with increasing ionic strength. It is shown that these reductions can be partially alleviated by replacing chloride with acetate anions.

Application of solution equilibrium analysis to **in vitro** RNA **transcription**.
Solution equilibrium analysis of **in vitro** RNA **transcription** has been applied to examine changes in pH, free magnesium concentration, and concentrations of all...

... magnesium which was determined to have a molar composition of 2:1 of Mg:PPi. **Transcription** rate and **efficiency** of NTP incorporation are also reduced with increasing ionic strength. It is shown that these...

6/3,K,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11108311 97402535 PMID: 9254702
Inhibition of transcription by the TAR RNA of HIV-1 in a nuclear extract of HeLa cells.
Yamamoto R; Koseki S; Ohkawa J; Murakami K; Nishikawa S; Taira K; Kumar P K
National Institute of Bioscience and Human Technology, AIST, MITI, Tsukuba Science City, Ibaraki 305, Japan.
Nucleic acids research (ENGLAND) Sep 1 1997, 25 (17) p3445-50, ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Regulation of transcription of human immunodeficiency virus type-1 (HIV-1) requires specific interaction of Tat protein with the trans-activation response region (TAR). Inhibition of replication of HIV-1 has previously been achieved with a TAR decoy, namely a short RNA oligonucleotide that corresponded to the sequence of the authentic TAR RNA. Since TAR RNA has the potential to interact with cellular factors, we examined the effect of TAR RNA on **efficiency** of **transcription** in nuclear of HeLa cell extracts. We performed an **in vitro** **transcription** assay in the presence of authentic TAR RNA using a template that was driven by the CMV (cytomegalovirus) early promoter in a HeLa nuclear extract and found, for the first time, that TAR RNA inhibited transcription by approximately 60-70% independently of the Tat-TAR interaction. Furthermore, we evaluated inhibition of transcription by variants of TAR RNA and found that the TAR RNA loop, bases surrounding the loop, the triple base bulge and the 'lower' stem region of TAR RNA were responsible for the inhibition of transcription. Taken together, earlier reports on proteins that bind to TAR RNA and the present results suggest that integrity of TAR RNA is important for efficient binding to cellular transcription factors. As judged from the significant inhibition observed in this study, the TAR decoy might sequester transcription factors and thus it might potentially be able to inhibit transcription of housekeeping genes that are unrelated to Tat function.

... the potential to interact with cellular factors, we examined the effect of TAR RNA on **efficiency** of **transcription** in nuclear of HeLa cell extracts. We performed an **in vitro** **transcription** assay in the presence of authentic TAR RNA using a template that was driven by...

6/3,K,AB/8 (Item 8 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

11100458 97394359 PMID: 9252077
Cleavage of viral RNA and inhibition of viral translation by hepatitis C virus RNA-specific hammerhead ribozyme in vitro.
Ohkawa K; Yuki N; Kanazawa Y; Ueda K; Mita E; Sasaki Y; Kasahara A; Hayashi N
First Department of Medicine, Osaka University School of Medicine, Suita, Japan.
Journal of hepatology (DENMARK) Jul 1997, 27 (1) p78-84, ISSN 0168-8278 Journal Code: 8503886
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND/AIMS: A hammerhead ribozyme has been used as a new way to suppress specific gene expression. We designed hammerhead ribozymes directed against hepatitis C virus RNA, and investigated their cleavage efficiency and inhibitory effect on viral translation in vitro. METHODS: Three hammerhead ribozymes bearing different cleavage sites in the core region of hepatitis C virus RNA (genotype 1b) were designed in this study. Ribozymes and the target hepatitis C virus RNA were synthesized by **in** vitro **transcription**. The cleavage **efficiency** was evaluated by the ribozyme cleavage assay. The inhibitory effect of the ribozyme on viral translation was further studied by the viral translation inhibition assay. RESULTS: All ribozymes specifically cleaved the target RNA of 1217 bases at a physiological temperature in a dose-dependent manner, with the specific cleavage increasing with a longer incubation period. The target RNA was cleaved most efficiently by the ribozyme with the cleavage site located nearest to the initiation codon. In the viral translation inhibition assay, all ribozymes showed a significant inhibitory effect on viral translation. The ribozyme with the cleavage site located farthest from the initiation codon blocked viral translation most efficiently, and demonstrated almost 70 to 80% inhibition. For ribozymes with the T7 transcription terminator sequence, both the target RNA cleavage and the inhibition of viral translation tended to be achieved less efficiently by ribozymes with T7 terminator than by those without it. CONCLUSIONS: These findings suggest that ribozyme-mediated hepatitis C virus RNA cleavage may serve as a new strategy in the treatment of hepatitis C virus infection.

...designed in this study. Ribozymes and the target hepatitis C virus RNA were synthesized by **in** vitro **transcription**. The cleavage **efficiency** was evaluated by the ribozyme cleavage assay. The inhibitory effect of the ribozyme on viral...

6/3,K,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11023289 97376849 PMID: 9233620
Family-specific differences in **transcription efficiency** of Ig heavy chain promoters.
Buchanan K L; Smith E A; Dou S; Corcoran L M; Webb C F
Immunobiology and Cancer Research Program, Oklahoma Medical Research Foundation, Oklahoma City 73104, USA.
Journal of immunology (Baltimore, Md. - 1950) (UNITED STATES) Aug 1 1997, 159 (3) p1247-54, ISSN 0022-1767 Journal Code: 2985117R
Contract/Grant No.: AI08972; AI; NIAID; GM46462; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Murine Ig variable region heavy chain genes (V(H)) are grouped into families based on coding sequence homology. We observed that the accompanying promoter sequences were also conserved in a family-specific manner. Remarkably, no one has directly compared the transcription efficiencies of V(H) genes from different families. Using an **in vitro transcription** system, we found that transcription efficiencies of different V(H) promoters differed by as much as 70-fold. These differences could be attributed to variation in the octamer-heptamer and TATA sequences, as well as to the presence or absence of initiator elements. The J558 family promoter exhibited the highest level of transcription and specifically interacted with an Oct-1 dimer not bound by other V(H) promoters. These data suggest that differential transcription and regulation of V(H) promoters could occur in vivo. The increased **transcription efficiency** of the J558 promoter relative to other V(H) promoters also presents a possible explanation for the abundance of J558 sterile transcripts observed before V(H)DJ(H) rearrangement.

Family-specific differences in **transcription efficiency** of Ig heavy chain promoters.

... has directly compared the transcription efficiencies of V(H) genes from different families. Using an **in vitro transcription** system, we found that transcription efficiencies of different V(H) promoters differed by as much...

... that differential transcription and regulation of V(H) promoters could occur in vivo. The increased **transcription efficiency** of the J558 promoter relative to other V(H) promoters also presents a possible explanation...

6/3,K,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10816026 97105904 PMID: 8948652

In vitro transcription of a poly(dA) x poly(dT)-containing sequence is inhibited by interaction between the template and its transcripts.

Kiyama R; Oishi M

Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Japan. kiyamar@hgc.ims.u-tokyo.ac.jp

Nucleic acids research (ENGLAND) Nov 15 1996, 24 (22) p4577-83, ISSN 0305-1048 Journal Code: 0411011

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Transcription of poly(dA) x poly(dT)-containing sequences was investigated in vitro using plasmids carrying a (dA)34 x (dT)34 tract in the coding region of the lacZ gene. The **efficiency** of **transcription** of the (dT)34 sequence on the transcribing strand by Escherichia coli RNA polymerase was substantially lower (approximately 60%) than that of the (dA)34 sequence or of the control lacZ gene. Analysis of the transcription process of the (dT)34 sequence by T3 RNA polymerase showed that the transcription was frequently arrested or terminated at the middle as well as immediately proximal of the (dA)34 x (dT)34 tract, and it occurred more prominently following accumulation of transcription products. This inhibition was strongly enhanced by the addition of the oligonucleotide (dT)34 or poly(U) to the reaction mixture, while (dA)34 and the duplex (dA)34 x (dT)34 suppressed the inhibition. A similar transcriptional inhibition was also observed in transcription mediated by T7 RNA polymerase and eukaryotic RNA polymerase II. We also demonstrated RNA x DNA complex formation of the (dA)34 x (dT)34 tract with poly(U), but not with poly(A). These findings strongly suggest that poly(dT)-containing

template sequences interact and form a complex with its transcription products, possibly an RNA x DNA triplex, which blocks further transcription. This would explain the instability of the plasmids transcribing mRNAs with poly(U) but not poly(A) tracts and the underrepresentation of poly(U) but not poly(A) tracts in mRNAs.

In vitro transcription of a poly(dA) x poly(dT)-containing sequence is inhibited by interaction between the...
...dA)34 x (dT)34 tract in the coding region of the lacZ gene. The **efficiency** of **transcription** of the (dT)34 sequence on the transcribing strand by Escherichia coli RNA polymerase was...
?

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? ds

Set     Items   Description
S1      14244   IN(W)VITRO(2N)TRANSCRIPTION
S2     429380   EFFICIENCY
S3        733   S1 AND S2
S4       1046   TRANSCRIPTION(2N)EFFICIENCY
S5        182   S1 AND S4
S6        106   RD (unique items)
? s in(w)vitro(w)transcription (2n)efficiency
Processing
Processing
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Processing
Processing
Processing
Processing
        28981413  IN
         1371005  VITRO
          574669  TRANSCRIPTION
          429380  EFFICIENCY
      S7      29  IN(W)VITRO(W)TRANSCRIPTION (2N)EFFICIENCY
? rd
>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
      S8      15  RD (unique items)
? t s8/3,k,ab/1-15

 8/3,K,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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11100458   97394359   PMID: 9252077
  Cleavage of viral RNA and inhibition of viral translation by hepatitis C
virus RNA-specific hammerhead ribozyme in vitro.
  Ohkawa K; Yuki N; Kanazawa Y; Ueda K; Mita E; Sasaki Y; Kasahara A;
Hayashi N
  First Department of Medicine, Osaka University School of Medicine, Suita,
Japan.
  Journal of hepatology (DENMARK) Jul 1997, 27 (1) p78-84, ISSN
0168-8278   Journal Code: 8503886
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  BACKGROUND/AIMS: A hammerhead ribozyme has been used as a new way to
suppress specific gene expression. We designed hammerhead ribozymes
directed against hepatitis C virus RNA, and investigated their cleavage
efficiency and inhibitory effect on viral translation in vitro. METHODS:
Three hammerhead ribozymes bearing different cleavage sites in the core
region of hepatitis C virus RNA (genotype 1b) were designed in this study.
Ribozymes and the target hepatitis C virus RNA were synthesized by in
 vitro transcription. The cleavage efficiency was
evaluated by the ribozyme cleavage assay. The inhibitory effect of the
ribozyme on viral translation was further studied by the viral translation
inhibition assay. RESULTS: All ribozymes specifically cleaved the target
RNA of 1217 bases at a physiological temperature in a dose-dependent
manner, with the specific cleavage increasing with a longer incubation
period. The target RNA was cleaved most efficiently by the ribozyme with
the cleavage site located nearest to the initiation codon. In the viral
translation inhibition assay, all ribozymes showed a significant inhibitory
effect on viral translation. The ribozyme with the cleavage site located
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farthest from the initiation codon blocked viral translation most efficiently, and demonstrated almost 70 to 80% inhibition. For ribozymes with the T7 transcription terminator sequence, both the target RNA cleavage and the inhibition of viral translation tended to be achieved less efficiently by ribozymes with T7 terminator than by those without it. CONCLUSIONS: These findings suggest that ribozyme-mediated hepatitis C virus RNA cleavage may serve as a new strategy in the treatment of hepatitis C virus infection.

...designed in this study. Ribozymes and the target hepatitis C virus RNA were synthesized by **in vitro transcription**. The cleavage **efficiency** was evaluated by the ribozyme cleavage assay. The inhibitory effect of the ribozyme on viral...

8/3,K,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10405497 96211495 PMID: 8648697
Cellular or viral protein binding to a cytomegalovirus promoter transcription initiation site: effects on transcription.
Macias M P; Huang L; Lashmit P E; Stinski M F
Department of Microbiology, College of Medicine, University of Iowa, Iowa City 52242, USA.
Journal of virology (UNITED STATES) Jun 1996, 70 (6) p3628-35, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: AI-13562; AI; NIAID; HL07638; HL; NHLBI; HL37121; HL; NHLBI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have previously shown that the IE2 protein of human cytomegalovirus (CMV) represses its own synthesis by binding to the major immediate-early promoter (M. P. Macias and M. F. Stinski, Proc. Natl. Acad. Sci. USA 90:707-711, 1993). The binding of a viral protein (IE2) and a cellular protein in the region of the transcription start site was investigated by site-specific mutational analysis and electrophoretic mobility shift assay. The viral protein and the cellular protein require different but adjacent core DNA sequence elements for binding. In situ chemical footprinting analysis of DNA-protein interactions with purified CMV IE2 protein or HeLa cell nuclear extracts demonstrated binding sites that overlap the transcription start site. The IE2 protein footprint was between bp -15 and +2, relative to the transcription start site, and the cellular protein was between bp -16 and +7. The ability of the unknown human cellular protein of approximately 150 kDa to bind the CMV major immediate-early promoter correlates with an increase in the level of transcription efficiency. Mutations in the core DNA sequence element for cellular protein binding significantly reduced the level of **in vitro transcription efficiency** . Mutations upstream and downstream of the core sequence moderately reduced the transcription efficiency level. Negative autoregulation of the CMV promoter by the viral IE2 protein may involve both binding to the DNA template and interference with the function of a cellular protein that binds to the transcription start site and enhances transcription efficiency.

... in the core DNA sequence element for cellular protein binding significantly reduced the level of **in vitro transcription efficiency** . Mutations upstream and downstream of the core sequence moderately reduced the transcription efficiency level. Negative...

8/3,K,AB/3 (Item 3 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

10350143 96152654 PMID: 8565070
Facilitated recycling pathway for RNA polymerase III.
Dieci G; Sentenac A
Service de Biochimie et Genetique Moleculaire Commissariat a l'Energie Atomique-Saclay, Gif-sur-Yvette, France.
Cell (UNITED STATES) Jan 26 1996, 84 (2) p245-52, ISSN 0092-8674 Journal Code: 0413066
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We show that the high **in vitro transcription efficiency** of yeast RNA pol III is mainly due to rapid recycling. Kinetic analysis shows that RNA polymerase recycling on preassembled tDNA.TFIIIC.TFIIIB complexes is much faster than the initial transcription cycle. High efficiency of RNA pol III recycling is favored at high UTP concentrations and requires termination at the natural termination signal. Runoff transcription does not allow efficient recycling. The reinitiation process shows increased resistance to heparin as compared with the primary initiation cycle, as if RNA polymerase was not released after termination. Indeed, template competition assays show that RNA pol III is committed to reinitiate on the same gene. A model is proposed where the polymerase molecule is directly transferred from the termination site to the promoter.

We show that the high **in vitro transcription efficiency** of yeast RNA pol III is mainly due to rapid recycling. Kinetic analysis shows that...

8/3,K,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09971316 21895943 PMID: 11897807
Improvement of non-radioactive in situ hybridization in human airway tissues: use of PCR-generated templates for synthesis of probes and an antibody sandwich technique for detection of hybridization.
Divjak Maja; Glare Eric M; Walters E Haydn
Department of Respiratory Medicine, Alfred Hospital, Monash University Medical School, Prahran, Victoria 3181, Australia. Maja.Divjak@med.monash.edu.au
journal of histochemistry and cytochemistry - official journal of the Histochemistry Society (United States) Apr 2002, 50 (4) p541-8, ISSN 0022-1554 Journal Code: 9815334
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We describe the use of non-traditional methods of probe synthesis and quantification and detection of hybridization that appreciably improved non-radioactive in situ hybridization (ISH) in human airway tissue. To avoid the problems of bacterial cloning, plasmid digestion, and probe hydrolysis, we synthesised complementary RNA probes (riboprobes) for ISH from PCR-generated DNA. DNA template was produced by nested PCR incorporation of T7 and SP6 RNA polymerase promoters. We then compared the **efficiency** of **in vitro transcription** from PCR-generated template with traditional plasmid template by quantifying the relative probe fluorescence in denaturing gels. Transcription with SP6 or T7 polymerase in either orientation produced TNF riboprobes from a single PCR-generated template more efficiently than from plasmid, providing there were no primer hairpin loops. Fluorescence quantification enabled equal

amounts of probe label to be used in ISH, eliminating signals from the sense probe and demonstrating that probes transcribed from PCR templates were as sensitive as hydrolyzed probe transcribed from plasmid. Detection of ISH by a conventional anti-hapten, alkaline phosphatase-based technique was found to cause tissue damage due to extended substrate incubation at high pH. We therefore developed a four-layer, avidin-biotin-peroxidase technique that afforded greater sensitivity, allowing brief substrate incubation and resulting in structural preservation of tissue.

...by nested PCR incorporation of T7 and SP6 RNA polymerase promoters. We then compared the **efficiency** of **in vitro transcription** from PCR-generated template with traditional plasmid template by quantifying the relative probe fluorescence in...

8/3,K,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08487144 95175360 PMID: 7870582
Molecular recognition of tRNA(Pro) by Escherichia coli proline tRNA synthetase in vitro.
Liu H; Peterson R; Kessler J; Musier-Forsyth K
Department of Chemistry, University of Minnesota, Minneapolis 55455.
Nucleic acids research (ENGLAND) Jan 11 1995, 23 (1) p165-9, ISSN 0305-1048 Journal Code: 0411011
Contract/Grant No.: GM49928; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
In this study, we identify a subset of nucleotides that specify aminoacylation of tRNA(Pro) by Escherichia coli proline tRNA synthetase in vitro. Twenty-two tRNA(Pro) variants were prepared by **in vitro transcription** and their **efficiency** of aminoacylation with proline (kcat/KM) was measured. From this analysis, we conclude that recognition elements for tRNA(Pro) aminoacylation by ProRS are located in at least three domains of the tRNA molecule. The largest decreases in the kinetic parameters for aminoacylation resulted from single substitutions at position G72 of the acceptor stem and position G36 of the anticodon. Anticodon nucleotide G35 and position A73 in the acceptor stem were also identified as major recognition elements. Moreover, bases that are believed to be important for maintaining the tertiary structure of the tRNA (G15 and C48) appear to be important for efficient recognition of tRNA(Pro) by ProRS in vitro.

... Escherichia coli proline tRNA synthetase in vitro. Twenty-two tRNA(Pro) variants were prepared by **in vitro transcription** and their **efficiency** of aminoacylation with proline (kcat/KM) was measured. From this analysis, we conclude that recognition...

8/3,K,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07664386 93119629 PMID: 1476730
Synthesis of cRNA probes from PCR-generated DNA.
Logel J; Dill D; Leonard S
Denver Veterans Administration Medical Center.
BioTechniques (UNITED STATES) Oct 1992, 13 (4) p604-10, ISSN 0736-6205 Journal Code: 8306785
Contract/Grant No.: MH44212; MH; NIMH

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

We have compared RNA polymerase promoter activities in PCR-generated DNA fragments for use in the in vitro transcription of cRNA probes. Sense oligonucleotide primers, specific for the mouse acidic fibroblast growth factor gene, were synthesized with 5' extensions containing promoter sequences for the T7, T3 and SP6 RNA polymerase promoters. A common antisense primer was used with each of the promoter/aFGF primers to prepare PCR-generated DNA fragments (minigenes). **In vitro transcription efficiency** for each of these constructs was evaluated by incorporation of radioactivity into the cRNA products. We find that both the T7 and T3 promoters can direct the synthesis of cRNA probes of high specific activity from a PCR-generated DNA fragment, but that SP6 cannot. No detectable cRNA product was obtained using either T7 polymerase on the T3/minigene or T3 on the T7/minigene. Antisense cRNA probes, transcribed from minigene constructs were used for both Northern and in situ hybridization studies. A PCR-generated DNA fragment with RNA polymerase promoter sequences at each end provides a single template for synthesis in vitro of either sense or antisense cRNA probes.

... used with each of the promoter/aFGF primers to prepare PCR-generated DNA fragments (minigenes). **In vitro transcription efficiency** for each of these constructs was evaluated by incorporation of radioactivity into the cRNA products...

8/3,K,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07013550 91254303 PMID: 1710452

Interaction of RNA polymerase II with acetylated nucleosomal core particles.

Pineiro M; Gonzalez P J; Hernandez F; Palacian E
Centro de Biologia Molecular, Universidad Autonoma de Madrid, Spain.

Biochemical and biophysical research communications (UNITED STATES) May 31 1991, 177 (1) p370-6, ISSN 0006-291X Journal Code: 0372516
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Chemical acetylation of nucleosomal cores is accompanied by an increase in their **efficiency** as **in vitro transcription** templates. Low amounts of acetic anhydride cause preferential modification of the amino-terminal tails of core histones. Modification of these domains, which causes moderate structural effects, is apparently correlated with the observed stimulation of RNA synthesis. In contrast, extensive modification of the globular regions of core histones, which is accompanied by a large structural relaxation of the particle, causes little additional effect on transcription. Acetylation of the amino-terminal domains of histones might stimulate transcription by changing the interaction of the histone tails with components of the transcriptional machinery.

Chemical acetylation of nucleosomal cores is accompanied by an increase in their **efficiency** as **in vitro transcription** templates. Low amounts of acetic anhydride cause preferential modification of the amino-terminal tails of...

8/3,K,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06848215 91088281 PMID: 2263463
Preparation and characterization of yeast nuclear extracts for efficient RNA polymerase B (II)-dependent transcription in vitro.
Verdier J M; Stalder R; Roberge M; Amati B; Sentenac A; Gasser S M
Centre d'Etudes Nucleaires de Saclay, Service de Biochimie, Gif-sur-Yvette, France.
Nucleic acids research (ENGLAND) Dec 11 1990, 18 (23) p7033-9, ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We present a reproducible method for the preparation of nuclear extracts from the yeast Saccharomyces cerevisiae that support efficient RNA polymerase B (II)-dependent transcription. Extracts from both a crude nuclear fraction and Percoll-purified nuclei are highly active for site-specific initiation and transcription of a G-free cassette under the Adenovirus major late promoter. At optimal extract concentrations transcription is at least 5 times more efficient with the yeast extracts than with HeLa whole cell extracts. We show that the transcriptional activity is sensitive to alpha-amanitin and to depletion of factor(s) recognizing the TATA-box of the promoter. The in vitro reaction showed maximal activity after 45 min, was very sensitive to Cl-, but was not affected by high concentrations of potassium. We find that the **efficiency** of **in vitro transcription** in nuclear extracts is reproducibly high when spheroplasting is performed with a partially purified beta 1,3-glucanase (lyticase). Therefore a simplified method to isolate the lyticase from the supernatant of Oerskovia xanthineolytica is also presented.

... to Cl-, but was not affected by high concentrations of potassium. We find that the **efficiency** of **in vitro transcription** in nuclear extracts is reproducibly high when spheroplasting is performed with a partially purified beta...

8/3,K,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06633170 90258740 PMID: 1692951
Differential in vitro transcription from the promoter of a rat alpha 2u globulin gene in liver and spleen nuclear extracts.
Sierra F; Tamone F; Mueller C R; Schibler U
Nestle Research Center, Vers-chez-les-Blanc, Lausanne, Switzerland.
Molecular biology & medicine (ENGLAND) Apr 1990, 7 (2) p131-46, ISSN 0735-1313 Journal Code: 8403879
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
When used in an in vitro transcription assay, the promoter of a cloned alpha 2u globulin gene is much more active in liver than in spleen nuclear extracts. Promoter deletion experiments suggest that both positive and negative regulatory mechanisms may be involved in the differential in vitro transcription from the alpha 2u globulin promoter in these two nuclear extracts. Interestingly, removal of promoter elements upstream from position -74 results in a significant increase of in vitro transcription in spleen but not in liver nuclear extracts, and thus reduces the difference in transcription observed with longer alpha 2u promoters in these two extracts. Deletion of additional nucleotides to position -43 strongly reduces the **in vitro transcription efficiency** of the promoter in extracts from both tissues. None of the examined promoters

containing between 3000 and 22 nucleotides of 5' flanking regions are differentially transcribed in liver nuclear extracts from either male or female rats. Thus, in contrast to cell-type specificity, sex-specificity could not be observed in our in vitro transcription experiments. DNase I protection experiments with crude nuclear extracts and partially or highly purified nuclear proteins suggests the presence of six recognition sites for DNA-binding factors between the TATA element and position -210. Some of these factors could be identified as proteins that also bind to elements within the albumin gene promoter.

...promoters in these two extracts. Deletion of additional nucleotides to position -43 strongly reduces the **in vitro transcription efficiency** of the promoter in extracts from both tissues. None of the examined promoters containing between...

8/3,K,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06596530 90221881 PMID: 2109308

Saturation mutagenesis of the Drosophila tRNA(Arg) gene B-Box intragenic promoter element: requirements for transcription activation and stable complex formation.

Gaeta B A; Sharp S J; Stewart T S
School of Biochemistry, University of New South Wales, Kensington, Australia.

Nucleic acids research (ENGLAND) Mar 25 1990, 18 (6) p1541-8, ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Transcription of eukaryotic tRNA genes is dependent on the A- and B-Box internal control regions (ICRs) and the upstream transcription modulatory region. The B-Box ICR spans nucleotides 52 to 62 and directs the primary binding of transcription factor C as the first step in the formation of a transcription complex. The conservation of the sequence of the B-Box in all tRNA species reflects its importance in both the expression of the gene and the processing, structure and function of the gene product. In order to identify the nucleotides essential to the promoter function of the B-Box ICR, site-directed mutagenesis was used to generate all the possible single point mutations at positions 52 to 58, 61 and 62 of a Drosophila melanogaster tRNA(Arg) gene. The effect of these mutations on gene transcription was evaluated using in vitro transcription and template exclusion competition assays. Optimal activity was displayed by the wild type tDNA(Arg) B-Box sequence but several other sequences supported in vitro transcription at wild type levels. The majority of mutants, however, showed lower **efficiency** in the **in vitro transcription** assay. Of the single point mutations, those at positions 53, 55, and 56 had a critical effect on gene function in Drosophila and HeLa transcription extracts and transcription factor interaction most likely requires base contacts at these positions. Since the effect of several of the point mutations cannot be explained in terms of possible major or minor groove contributions the possibility is raised that local DNA geometry also is an important determinant in specifying B-Box function.

... supported in vitro transcription at wild type levels. The majority of mutants, however, showed lower **efficiency** in the **in vitro transcription** assay. Of the single point mutations, those at positions 53, 55, and 56 had a...

8/3,K,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05893458 88247755 PMID: 3260027
In vitro transcription and translational **efficiency** of chimeric SP6 messenger RNAs devoid of 5' vector nucleotides.
Jobling S A; Cuthbert C M; Rogers S G; Fraley R T; Gehrke L
Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA 02139.
Nucleic acids research (ENGLAND) May 25 1988, 16 (10) p4483-98, ISSN 0305-1048 Journal Code: 0411011
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A plasmid containing the bacteriophage SP6 promoter, designated pHSTO, permits in vitro transcription of RNAs devoid of vector-derived nucleotides. This vector has been characterized for relative transcriptional activity using constructs which alter the conserved nucleotides extending beyond the SP6 transcriptional initiation site. SP6 polymerase efficiently transcribes cDNA inserts which contain a guanosine (G) nucleotide at position +1 relative to the SP6 promoter; however, inserts with an adenosine (A) or pyrimidine at position +1 are not transcribed. Several cellular and viral cDNAs have been transcribed into translatable messenger RNA using this vector; however, SP6 polymerase will not transcribe the A-T rich untranslated leader from alfalfa mosaic virus RNA 4 efficiently unless the viral mRNA cap site is separated from the transcriptional initiation site by twelve base pairs of vector DNA. Chimeric messenger RNAs were created by linking the untranslated leader sequence of several viral mRNAs to the coding region of barley alpha-amylase, and the resultant mRNAs were translated in a wheat germ extract to determine relative translational efficiencies. The untranslated leader sequences of turnip yellow mosaic virus coat protein mRNA and black beetle virus RNA 2 did not increase translational efficiency, while the tobacco mosaic virus leader stimulated translation significantly. The results indicate that substitution of a cognate untranslated leader sequence with a leader derived from a highly efficient mRNA does not necessarily predict enhanced translational efficiency of the chimeric mRNA.

In vitro transcription and translational **efficiency** of chimeric SP6 messenger RNAs devoid of 5' vector nucleotides.

8/3,K,AB/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

04925297 85232066 PMID: 3924738
Drosophila melanogaster tRNAVal3b genes and their allogenes.
Leung J; Addison W R; Delaney A D; MacKay R M; Miller R C; Spiegelman G B ; Grigliatti T A; Tener G M
Gene (NETHERLANDS) 1985, 34 (2-3) p207-17, ISSN 0378-1119 Journal Code: 7706761
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Drosophila tRNAVal3b genes have been analyzed with respect to their nucleotide sequence and **in vitro transcription efficiency** . Plasmid pDt78R contains a single tRNA gene derived from the major tRNAVal3b gene cluster at chromosome band 84D. Its sequence

corresponds to that of the tRNAVal3b. Two other plasmids, pDt41R and pDt48, each contain a tRNAVal3b-like gene from the minor tRNAVal3b gene cluster at chromosome bands 90BC. They contain the expected CAC anticodon, but their sequence differs from the tRNA at four positions. In homologous cell-free extracts, the tRNAVal3b variant genes in pDt41R and pDt48 are transcribed an order of magnitude more efficiently than the tRNAVal3b gene in pDt78R. However, the variant genes do not appear to contribute significantly to the in vivo tRNA pool [Larsen et al.: Mol. Gen. Genet. 185 (1982) 390-396]. We propose the term allogenes to describe families of related DNA sequences that may code for variant forms of a standard tRNA isoaccepting species.

Drosophila tRNAVal3b genes have been analyzed with respect to their nucleotide sequence and **in vitro transcription efficiency** . Plasmid pDt78R contains a single tRNA gene derived from the major tRNAVal3b gene cluster at...

8/3,K,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

04893151 85199825 PMID: 3994989
Efficiency of **in vitro transcription** of Dictyostelium discoideum actin gene is affected by the nucleotide sequence of the transcription initiation region.
Takiya S; Takahashi K; Iwabuchi M; Suzuki Y
Biochemistry (UNITED STATES) Feb 12 1985, 24 (4) p1040-7, ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The actin gene of Dictyostelium discoideum is transcribed faithfully but with very low efficiency in a cell-free system containing HeLa cell extract [Takiya, S., Tabata, T., Iwabuchi, M., Hirose, S., & Suzuki, Y. (1984) J. Biochem. (Tokyo) 95, 1367-1377]. Using the same in vitro system, we determined that the promoter activity of the actin 5 gene is 100-200 times weaker than that of the silkworm fibroin gene. To clarify the cause of the low transcription efficiency, various chimeric genes were constructed from the actin and fibroin genes, and their transcription efficiencies were examined in vitro. Both the TATA box and the transcription initiation site of the two natural genes functioned in the transcription of the chimeric genes, the efficiency of which was especially affected by the transcription initiation region. In chimeric genes having the upstream sequence of the actin gene and a downstream sequence including the transcription initiation site of the fibroin gene, the transcription efficiency was higher than one-third of that of the natural fibroin gene. In chimeric genes having the actin transcription initiation region and an upstream sequence of the fibroin gene, the transcription efficiency was as low as that of the natural actin gene. We concluded that the transcription initiation site is a part of the promoter and an essential region for directing faithful and efficient initiation of gene transcription.

Efficiency of **in vitro transcription** of Dictyostelium discoideum actin gene is affected by the nucleotide sequence of the transcription initiation...

8/3,K,AB/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

04828543 85134872 PMID: 6098685
A transcription enhancer acts in vitro over distances of hundreds of

base-pairs on both circular and linear templates but not on chromatin-reconstituted DNA.
Sergeant A; Bohmann D; Zentgraf H; Weiher H; Keller W
Journal of molecular biology (ENGLAND) Dec 15 1984, 180 (3) p577-600
, ISSN 0022-2836 Journal Code: 2985088R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have analyzed the effect of nucleosome formation and of the simian virus (SV40) enhancer on the **efficiency** of **in vitro transcription** . In a whole cell extract made from HeLa cells, nucleosome assembly on DNA results in the formation of chromatin-like complexes. However, transcription was detectable only when the DNA templates were partially or totally depleted of nucleosomes. On nucleosome-free templates, when the SV40 enhancer was present upstream from the complete SV40 early or rabbit beta-globin promoters, there was a five- to tenfold stimulation of specific transcription. When present upstream from its homologous promoter, the SV40 enhancer activated SV40 early transcription independently of its orientation with respect to the coding sequence. Point mutations known to impair the SV40 enhancer function in vivo had a similar effect in vitro. The extent of the enhancing effect was the same with linear or circular templates. When the SV40 enhancer was inserted upstream from the rabbit beta-globin gene, the activation of transcription was reduced with increasing distance between the enhancer and beta-globin upstream promoter elements, but was still significant over a distance of more than 400 base-pairs.

... analyzed the effect of nucleosome formation and of the simian virus (SV40) enhancer on the **efficiency** of **in vitro transcription** . In a whole cell extract made from HeLa cells, nucleosome assembly on DNA results in...

8/3,K,AB/15 (Item 1 from file: 55)
DIALOG(R)File 55:Biosis Previews(R)

08456212 BIOSIS NO.: 199344006212
Reduced **efficiency** of **in vitro transcription** in a G-tailed cDNA insert.
AUTHOR: Shanbaky N M; Pressley T A
AUTHOR ADDRESS: Dep. Physiol. Cell Biol., Univ. Texas Med. Sch., Houston, Tex. 77225**Denmark
JOURNAL: Molecular Biology of the Cell 3 (SUPPL.):p89A 1992
CONFERENCE/MEETING: Thirty-second Annual Meeting of the American Society for Cell Biology, Denver, Colorado, USA, November 15-19, 1992. MOL BIOL CELL
ISSN: 1059-1524
RECORD TYPE: Citation
LANGUAGE: English
1992

Reduced **efficiency** of **in vitro transcription** in a G-tailed cDNA insert.
?

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      ---  -----  -----------
? s crna(2n)standard??
             54  CRNA
          71080  STANDARD??
      S1      1  CRNA(2N)STANDARD??
? t s1/3,k,ab/1

 1/3,K,AB/1
DIALOG(R)File 340:CLAIMS(R)/US Patent
(c) 2003 IFI/CLAIMS(R). All rts. reserv.

Dialog Acc No: 2673543  IFI Acc No: 9531052
Document Type: C
QUANTITATION OF NUCLEIC ACIDS USING THE POLYMERASE CHAIN REACTION; PLASMID
FOR USE AS INTERNAL STANDARD
Inventors: Doyle Michael V (US); Mark David F (US); Wang Alice M (US)
Assignee: Hoffmann-La Roche Inc
Assignee Code: 39424  Document Type: REASSIGNED
Publication (No,Date), Applic (No,Date):
US 5476774     19951219 US 9328464     19930309
Publication Kind:  A
Calculated Expiration: 20121219
(Cited in 007 later patents)  Document Type: CERTIFICATE OF CORRECTION
Certificate of Correction Date: 19960507
Continuation Pub(No),Applic(No,Date): US 5219727                US 89413623
  19890928
Cont.-in-part Pub(No),Applic(No,Date): ABANDONED                 US
89396986     19890821
Priority Applic(No,Date): US 9328464     19930309; US 89413623     19890928;
US 89396986     19890821
Disclaimer Date: 20100615
Abstract: The present invention provides a method for determining the
amount of a target acid segment in a sample by polymerase chain reaction.
The method involves the simultaneous amplification or the target nucleic
acid segment and an internal standard nucleic acid segment. The amount of
amplified DNA from each segment is determined and compared to standard
curves to determine the amount of the target nucleic acid segment present
in the sample prior to amplification. The method is especially preferred
for determining the quantity of a specific mRNA species in a biological
sample. Additionally, an internal standard is provided useful for
quantitation of multiple mRNA species.


Non-exemplary Claims: ...12. The kit of claim 6, wherein said internal
   standard  is a cRNA molecule...

...present in a biological sample, said reaction mixture comprising a
   predetermined initial amount of internal standard cRNA, a
   target RNA, and a target-specific primer for initiating cDNA synthesis,
   wherein said primer can serve to initiate reverse transcription of a
   nucleic acid segment contained within said standard cRNA
   together with a segment contained within the particular target nucleic
   acid, and wherein said standard...
?
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? s cRNA
      S1     5623  CRNA
? s transcription(5n)efficiency
           574756  TRANSCRIPTION
           429403  EFFICIENCY
      S2     1408  TRANSCRIPTION(5N)EFFICIENCY
? s s1 and s2
             5623  S1
             1408  S2
      S3       15  S1 AND S2
? rd
>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
      S4        7  RD (unique items)
? t s4/3,k,ab/1-7

 4/3,K,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

11232969   98110313   PMID: 9448846
   A method for preventing artifactual binding of cRNA probes to
neurons caused by in situ hybridization.
  Blodorn B; Bruck W; Rieckmann P; Felgenhauer K; Mader M
  Analytical biochemistry (UNITED STATES)  Jan 1 1998,  255  (1)  p95-100,
ISSN 0003-2697   Journal Code: 0370535
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  When in situ hybridization was used for the detection of mRNA for the
beta-trace protein (beta-trace; prostaglandin-D-synthase) in sections of
rat and porcine brains, unspecific binding reactions of sense and antisense
probes to neurons were observed. The beta-trace fragment which served as a
template for the synthesis of cRNA probes was blunt end-cloned in the
vector pCR-Script SK (+). It was demonstrated that the unspecific signals
were caused by artifactual binding of two portions of the cRNA which
correspond to sequences of the multicloning site of this vector. These
sequences are localized between the SrfI restriction site (or the insert)
and the promoter for the T7 RNA polymerase. Thus, artifactual binding could
be prevented using riboprobes synthesized by T3 RNA polymerase instead of
T7 RNA polymerase. Because of the relatively weak transcription
efficiency of T3 RNA polymerase, as compared with T7 RNA polymerase,
a blocking procedure was established which allowed successful in situ
hybridization with T7 RNA polymerase-synthesized probes. Blocking was
performed using synthetic oligonucleotides deduced from the two sequences
of the multicloning site which were found to be responsible for artifactual
binding.

   A method for preventing artifactual binding of cRNA probes to
neurons caused by in situ hybridization.
  ... were observed. The beta-trace fragment which served as a template for
the synthesis of cRNA probes was blunt end-cloned in the vector
pCR-Script SK (+). It was demonstrated that the unspecific signals were
caused by artifactual binding of two portions of the cRNA which
correspond to sequences of the multicloning site of this vector. These
sequences are localized...

... synthesized by T3 RNA polymerase instead of T7 RNA polymerase. Because
of the relatively weak transcription efficiency of T3 RNA
polymerase, as compared with T7 RNA polymerase, a blocking procedure was
```

established...

4/3,K,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08502702 95190978 PMID: 7884855
Changes in the viral mRNA expression pattern correlate with a rapid rate of CD4+ T-cell number decline in human immunodeficiency virus type 1-infected individuals.
Furtado M R; Kingsley L A; Wolinsky S M
Department of Pathology, Northwestern University Medical School, Chicago, Illinois 60611.
Journal of virology (UNITED STATES) Apr 1995, 69 (4) p2092-2100, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: AI 32535; AI; NIAID; AI 45218; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The rate of disease progression varies considerably among human immunodeficiency virus type 1 (HIV-1)-infected individuals. Several cross-sectional studies have shown an association between the stage of HIV-1 disease and the viral burden or the relative levels of viral gene expression. To study the extent of HIV-1 transcription and replication and its correlations with disease progression, we quantified serial, longitudinal samples of blood cells from 10 HIV-1-infected individuals with markedly different rates of CD4+ T-cell number decline following seroconversion. After normalization for the input nucleic acid content, multiply spliced viral mRNA and unspliced viral RNA were quantified by competitive reverse transcription-PCR using oligonucleotide primers which flank the major tat/rev/nef splice junction and span an internal region of the gag open reading frame, respectively. Coamplification of internal **cRNA** template controls was used to normalize for variation in the **efficiency** of reverse **transcription** and in vitro enzymatic amplification. Similarly, proviral DNA was also quantified by competitive PCR performed within the linear range of amplification. Viral RNA was detected in the blood cells of each individual from all time points regardless of the rate of CD4+ T-cell decline. Unspliced genomic viral RNA rapidly increased in the blood cells from HIV-1-infected individuals who had a precipitously declining CD4+ T-cell number. In contrast, both unspliced and multiply spliced viral mRNAs remained relatively stable in the blood cells from HIV-1-infected individuals who have had a relatively benign clinical course. These data demonstrate that the extent of viral transcription and replication correlates with the rate of CD4+ T-cell number decline and that quantifying intracellular viral RNA is of potential prognostic value.

... and span an internal region of the gag open reading frame, respectively. Coamplification of internal **cRNA** template controls was used to normalize for variation in the **efficiency** of reverse **transcription** and in vitro enzymatic amplification. Similarly, proviral DNA was also quantified by competitive PCR performed...

4/3,K,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07951281 94016814 PMID: 8411337
In vitro expression of the human cytomegalovirus DNA polymerase gene: effects of sequence alterations on enzyme activity.
Ye L B; Huang E S

Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill 27599-7295.
Journal of virology (UNITED STATES) Nov 1993, 67 (11) p6339-47, ISSN 0022-538X Journal Code: 0113724
Contract/Grant No.: AI12717; AI; NIAID; CA15036; CA; NCI; CA21773; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Genomic DNA of the Towne strain human cytomegalovirus polymerase (pol) gene (4.4-kb RsrII-NcoI segment of the EcoRI J fragment) was cloned into plasmids containing either the T3 or the T7 promoter for in vitro **transcription**-translation studies. The translation **efficiency** of unmodified pol **cRNA** was poor in this system and could not be improved by capping. However, the efficiency could be enhanced by replacing the leader sequence with a 40-bp AT-rich sequence derived from an alfalfa mosaic virus, R4. pol **cRNA** directed the synthesis of a 140-kDa polypeptide in a rabbit reticulocyte translation system. The in vitro-translated wild-type enzyme possessed significant polymerization activity which could be stimulated by salt as could that of the authentic enzyme purified from virus-infected cells. To study the critical domains of this enzyme, nine mutations were introduced into the pol gene around the conserved domains of eukaryotic polymerase by oligonucleotide-directed mutagenesis. Two constructs with mutations at amino acid residues 323 to 325 (M32QS) and 725 to 726 (M72II) remained active, with partial loss of enzyme activity, while the enzyme activities of other mutants with alterations at four domains located around amino acid residues 729 to 730 (M73HN), 804 to 807 (M80 and DE80), 910 to 913 (M91 and DE91), and 962 to 964 (M96 and DE96) were abolished. DNA template and triphosphate binding assays indicated that the mutation at 804 to 807 (conserved region III) lost the ability to bind DNA template, and four mutants, M73HN (within conserved region II), M80 (in region III), M91 (in region I), and M96 (around region V [962 to 964; amino acid sequence KKR]), failed to bind deoxyribonucleoside triphosphate. These data suggest that conserved region III is essential for DNA template binding, while residues between conserved region II and V (725 to 964) are involved in triphosphate binding.

... was cloned into plasmids containing either the T3 or the T7 promoter for in vitro **transcription**-translation studies. The translation **efficiency** of unmodified pol **cRNA** was poor in this system and could not be improved by capping. However, the efficiency...

... with a 40-bp AT-rich sequence derived from an alfalfa mosaic virus, R4. pol **cRNA** directed the synthesis of a 140-kDa polypeptide in a rabbit reticulocyte translation system. The...

4/3,K,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07735763 93191041 PMID: 8447366
Molecular cloning and quantification of sarcoplasmic reticulum Ca(2+)-ATPase isoforms in rat muscles.
Wu K D; Lytton J
Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts 02115.
American journal of physiology (UNITED STATES) Feb 1993, 264 (2 Pt 1) pC333-41, ISSN 0002-9513 Journal Code: 0370511
Contract/Grant No.: DK-42879; DK; NIDDK
Erratum in Am J Physiol 1994 Jul;267(1 Pt 1) section C followi
Document type: Journal Article
Languages: ENGLISH

Main Citation Owner: NLM
Record type: Completed
A cDNA encoding the full-length adult rat fast-twitch muscle Ca(2+)-adenosinetriphosphatase (ATPase) was cloned. The deduced amino acid sequence of this molecule has 97 and 90% identity with those of rabbit fast-twitch muscle and chicken skeletal muscle Ca(2+)-ATPases, respectively. Specific probes from the 3'-untranslated region of each sarcoplasmic or endoplasmic reticulum Ca(2+)-ATPase (SERCA) gene product and full-length **cRNA** transcript standards were used to determine the quantity of mRNA encoding each isoform in various rat muscles. Quantitative immunoblotting was also used to determine the protein content of each SERCA isoform. Fast-twitch fibers expressed both SERCA1 mRNA and protein at a level two- to fivefold higher than SERCA2 was expressed in slow-twitch fibers. We observed a protein-to-mRNA ratio that varied from approximately 500,000 molecules per molecule in the fast-twitch muscles to approximately 200,000 in cardiac and smooth muscles. There was no difference, however, between the ratio for different isoforms in the same muscle. The content of Ca2+ pump in a given muscle therefore depends on at least three factors: 1) the **efficiency** of gene **transcription** and message stability (fiber type dependent), 2) the efficiency of translation and protein stability (muscle identity dependent), and 3) fiber composition of the muscle.

... of each sarcoplasmic or endoplasmic reticulum Ca(2+)-ATPase (SERCA) gene product and full-length **cRNA** transcript standards were used to determine the quantity of mRNA encoding each isoform in various...

...Ca2+ pump in a given muscle therefore depends on at least three factors: 1) the **efficiency** of gene **transcription** and message stability (fiber type dependent), 2) the efficiency of translation and protein stability (muscle...

4/3,K,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07664386 93119629 PMID: 1476730
Synthesis of **cRNA** probes from PCR-generated DNA.
Logel J; Dill D; Leonard S
Denver Veterans Administration Medical Center.
BioTechniques (UNITED STATES) Oct 1992, 13 (4) p604-10, ISSN 0736-6205 Journal Code: 8306785
Contract/Grant No.: MH44212; MH; NIMH
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have compared RNA polymerase promoter activities in PCR-generated DNA fragments for use in the in vitro transcription of **cRNA** probes. Sense oligonucleotide primers, specific for the mouse acidic fibroblast growth factor gene, were synthesized with 5' extensions containing promoter sequences for the T7, T3 and SP6 RNA polymerase promoters. A common antisense primer was used with each of the promoter/aFGF primers to prepare PCR-generated DNA fragments (minigenes). In vitro **transcription efficiency** for each of these constructs was evaluated by incorporation of radioactivity into the **cRNA** products. We find that both the T7 and T3 promoters can direct the synthesis of **cRNA** probes of high specific activity from a PCR-generated DNA fragment, but that SP6 cannot. No detectable **cRNA** product was obtained using either T7 polymerase on the T3/minigene or T3 on the T7/minigene. Antisense **cRNA** probes, transcribed from minigene constructs were used for both Northern and in situ hybridization studies. A PCR-generated DNA fragment with RNA polymerase promoter sequences at each end provides a single

template for synthesis in vitro of either sense or antisense **cRNA** probes.

Synthesis of **cRNA** probes from PCR-generated DNA.
... promoter activities in PCR-generated DNA fragments for use in the in vitro transcription of **cRNA** probes. Sense oligonucleotide primers, specific for the mouse acidic fibroblast growth factor gene, were synthesized...

...each of the promoter/aFGF primers to prepare PCR-generated DNA fragments (minigenes). In vitro **transcription efficiency** for each of these constructs was evaluated by incorporation of radioactivity into the **cRNA** products. We find that both the T7 and T3 promoters can direct the synthesis of **cRNA** probes of high specific activity from a PCR-generated DNA fragment, but that SP6 cannot. No detectable **cRNA** product was obtained using either T7 polymerase on the T3/minigene or T3 on the T7/minigene. Antisense **cRNA** probes, transcribed from minigene constructs were used for both Northern and in situ hybridization studies...

... each end provides a single template for synthesis in vitro of either sense or antisense **cRNA** probes.

4/3,K,AB/6 (Item 1 from file: 55)
DIALOG(R)File 55:Biosis Previews(R)

13741880 BIOSIS NO.: 200200370701
Quantitative real-time RT-PCR using **cRNA** standards for the measurement of cytokine gene expression.
AUTHOR: Quinton Lee Joseph(a); Zhang Ping; Nelson Steve; Boe Darren M(a); Zhong Qiu; Kolls Jay K; Bagby Gregory J(a)
AUTHOR ADDRESS: (a)Physiology, LSU Health Sciences Center, 533 Bolivar St., New Orleans, LA, 70112**USA
JOURNAL: FASEB Journal 16 (5):pA1083-A1084 March 22, 2002
MEDIUM: print
CONFERENCE/MEETING: Annual Meeting of Professional Research Scientists on Experimental Biology New Orleans, Louisiana, USA April 20-24, 2002
ISSN: 0892-6638
RECORD TYPE: Abstract
LANGUAGE: English

4/9

ABSTRACT: Real-time RT-PCR has greatly enhanced the analysis of gene expression in many biological applications. Advantages of real-time RT-PCR have centered on its sensitivity and accurate quantification of nucleic acids. However, the ability of this technology to justly quantify mRNA expression is largely dependent on the standards to which unknowns are compared. Here we introduce a method for analyzing cytokine mRNA expression using **cRNA** standards. Use of **cRNA** template more accurately accounts for the **efficiency** of reverse **transcription**, which can be as low as 10%. In order to develop **cRNA** standards for the desired cytokines (rat TNF-alpha, rat MIP-2, rat CINC and murine G-CSF), corresponding cDNA sequences were cloned into the pCR2.1 vector, and subjected to in vitro transcription. Selected concentrations of the cytokine **cRNA** transcripts were then analyzed by real-time RT-PCR. The **cRNA** standard curves for TNF-alpha and G-CSF ranged from 102-109 copies **cRNA** (R2gtoreq.99). Likewise, the dynamic range for MIP-2 and CINC was 103-1010 copies **cRNA** (R2gtoreq.99). Therefore, the use of **cRNA** standards in conjunction with real-time RT-PCR may represent a more accurate approach to quantifying cytokine mRNA expression.

2002

Quantitative real-time RT-PCR using **cRNA** standards for the measurement of cytokine gene expression.

...ABSTRACT: which unknowns are compared. Here we introduce a method for analyzing cytokine mRNA expression using **cRNA** standards. Use of **cRNA** template more accurately accounts for the **efficiency** of reverse **transcription**, which can be as low as 10%. In order to develop **cRNA** standards for the desired cytokines (rat TNF-alpha, rat MIP-2, rat CINC and murine...

...the pCR2.1 vector, and subjected to in vitro transcription. Selected concentrations of the cytokine **cRNA** transcripts were then analyzed by real-time RT-PCR. The **cRNA** standard curves for TNF-alpha and G-CSF ranged from 102-109 copies **cRNA** (R2gtoreq.99). Likewise, the dynamic range for MIP-2 and CINC was 103-1010 copies **cRNA** (R2gtoreq.99). Therefore, the use of **cRNA** standards in conjunction with real-time RT-PCR may represent a more accurate approach to...
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: **cRNA** {complementary RNA...

4/3,K,AB/7 (Item 2 from file: 55)
DIALOG(R)File 55:Biosis Previews(R)

08820835 BIOSIS NO.: 199395110186
Molecular cloning and quantification of sarcoplasmic reticulum calcium-ATPase isoforms in rat muscles.
AUTHOR: Wu Kwan-Dun; Lytton Jonathan(a)
AUTHOR ADDRESS: (a)Renal Div., Brigham and Women's Hosp., 75 Francis St., Boston, MA 02115**USA
JOURNAL: American Journal of Physiology 264 (2 PART 1):pC333-C341 1993
ISSN: 0002-9513
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A cDNA encoding the full-length adult rat fast-twitch muscle Ca-2+-adenosinetriphosphatase (ATPase) was cloned. The deduced amino acid sequence of this molecule has 97 and 90% identity with those of rabbit fast-twitch muscle and chicken skeletal muscle Ca-2+-ATPase, respectively. Specific probes from the 3'-untranslated region of each sarcoplasmic or endoplasmic reticulum Ca-2+-ATPase (SERCA) gene product and full-length **cRNA** transcript standards were used to determine the quantity of mRNA encoding each isoform in various rat muscles. Quantitative immunoblotting was also used to determine the protein content of each SERCA isoform. Fast-twitch fibers expressed both SERCA1 mRNA and protein at a level two- to fivefold higher than SERCA2 was expressed in slow-twitch fibers. We observed a protein-to-mRNA ratio that varied from apprx 500,000 molecules per molecule in the fastr-twitch muscles to apprx 200,000 in cardiac and smooth muscles. There was no difference, however, between the ratio for different isoforms in the same muscle. The content of Ca-2+ pump in a given muscle therefore depends on at least three factors: 1) the **efficiency** of gene **transcription** and message stability (fiber type dependent), 2) the efficiency of translation and protein stability (muscle identity dependent), and 3) fiber composition of the muscle.

1993

...ABSTRACT: of each sarcoplasmic or endoplasmic reticulum Ca-2+-ATPase (SERCA) gene product and full-length **cRNA** transcript standards were used to determine the quantity of mRNA encoding each isoform in various ...

...2+ pump in a given muscle therefore depends on at least three factors:
1) the **efficiency** of gene **transcription** and message stability
(fiber type dependent), 2) the efficiency of translation and protein
stability (muscle...
?